New insights in the disinfection of the root canal system using different research models

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SUMMARY

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Biofilm in the root canal system is a great challenge during disinfection in the endodontic therapy, mainly the biofilm located in areas of anatomic complexities. **Chapter 1** gives a brief overview on how endodontic infection starts and continues its path through the root canal space, where the bacteria grow and function in a biofilm mode of life. Besides, it shows how the anatomic complexities of the root canal system can influence disinfection and the most common antimicrobials agents used in the endodontic therapy.

In this regard, **Chapter 2** focuses on the most common intracanal medication used between sessions of endodontic therapy: the calcium hydroxide paste. Thus, in this study we evaluated the intratubular decontamination ability of five different formulations of calcium hydroxide pastes against *Enterococcus faecalis* and their penetrability in dentine by Confocal Laser Scanning Microscopy. The physicochemical properties of the different calcium hydroxide pastes, such as pH, solubility and calcium ion release were evaluated using a pHmeter, the Micro CT and an atomic absorption spectrophotometer. For the manipulation of calcium hydroxide, distilled water and propylene glycol were used as vehicles. Besides, propylene glycol was used with three additives, ethanolic extract of propolis, chlorhexidine and camphorated paramonochlorophenol (CPMC). The paste with CPMC was significantly more antimicrobial when compared to the paste with distilled water. In general, the pastes withpropylene glycol as vehicle were more antimicrobial, penetrated better in the dentine tubules and had better physicochemical properties.

In **Chapter 3** we evaluate the antimicrobial efficacy of an innovative root canal irrigant with silver nanoparticles, in comparison with chlorhexidine and the gold standard sodium hypochlorite. The irrigants were tested for 5, 15 and 30 minutes on an *Enterococcus faecalis* biofilm grown on bovine dentine blocks and inside dentinal tubules of bovine teeth. The samples were analysed by Confocal Laser Scanning Microscopy showing that sodium hypochlorite showed the best antibiofilm effect, in also in the dentinal tubules, followed by chlorhexidine and the innovative irrigant with silver nanoparticles. The latter showed to be ineffective against *Enterococcus faecalis* in comparison with the other tested irrigants used in the endodontic therapy.

In **Chapter 4**, an Optical Coherence Tomography analysis of biofilm removal from polydimethylsiloxane root canal models with lateral morphological features was described for the first time. Sodium hypochlorite at various concentrations and in different flow rates were used for root canal irrigation followed by a final irrigation with buffer at a high flow rate. Besides, irrigant velocity profiles inside the root canal model were simulated using a validated method of Computational Fluid Dynamics. These flow velocities were correlated with biofilm removal. The irrigant flow rate had more influence on biofilm removal than the irrigant concentration. The irrigant velocity influenced biofilm removal since in areas with higher velocities more biofilm was removed. A high flow rate was also related with higher irrigant velocity inside the model.

In **Chapter 5** we presented a comparison of four different irrigation protocols regarding their ability to remove biofilm as well from a root canal model with lateral morphological features like an isthmus and lateral canal like structure as from human dentine tubules, using Optical Coherence Tomography or Confocal Laser Scanning Microscopy as evaluation method respectively. The irrigation protocols used were syringe irrigation with a buffer solution (control group), a modified salt solution called RISA, sodium hypochlorite and ultrasonic activation of the buffer solution followed by a final irrigation at a higher flow rate using the buffer solution. The mechanical effect of syringe irrigation showed to be a relevant factor to be observed when studying biofilm removal. Ultrasonic activation of the irrigant showed to be effective when the contact surface biofilm-irrigant was small. In the dentinal tubule model, besides the antimicrobial efficacy, a recolonization analysis was performed five days after the treatment. This analysis showed that the post treatment remaining biofilm was able to regrow inside the dentine tubules in a five days period without any extra nutrition. The antibiofilm effect of buffer solution, RISA and sodium hypochlorite was tested on a biofilm grown on dentine disks. The biofilm thickness reduction and biofilm viscoelastic properties of the post treatment remaining biofilm were evaluated, showing no difference among the studied substances.

In **Chapter 6** we further investigated the influence of the irrigant, flow rate, irrigant refreshment and exposure time on biofilm removal from the root canal models with the lateral morphological features by Optical Coherence Tomography. Sodium hypochlorite and demineralized water (control group) were used as irrigant solutions delivered in the root canal model with flow rate 0.05 or 0.1 mL/s. Sodium hypochlorite and a higher flow rate showed more biofilm removal from the isthmus like structures, whereas for the lateral canal the flow rate had no influence. Refreshment did not have a significant effect on biofilm removal. Analysis of the sequential refreshments in the same biofilm showed a cumulative effect of irrigation on the biofilm.

Based on the results obtained in the studies described in the previous chapters, **Chapter 7** discussed the findings, different biofilm models used and correlations among them. Also, it gives an overview of the future perspectives based on the performed studies in order to optimize disinfection procedures during endodontic therapy possibly leading an improvement in healing of apical periodontitis.

SAMENVATTING

Biofilm in het wortelkanaalsysteem is waarschijnlijk de grootste uitdaging tijdens de wortelkanaalbehandeling, vooral de biofilm die zich bevind in de moeilijk bereikbare anatomisch complexe gebieden zoals bijvoorbeeld een ovale uitloper of een isthmus tussen twee kanalen. **Hoofdstuk 1** geeft een kort overzicht over het verloop van de infectie van het wortelkanaalsysteem. Ook wordt er aandacht besteed aan de rol van de complexe anatomie tijdens de desinfectie en de meest voorkomende desinfectie procedures en middelen die tijdens de wortelkanaalbehandeling worden gebruikt.

In **Hoofdstuk 2** focust de aandacht zich op de meest voorkomende wortelkanaal medicatie die toegepast wordt indien een wortelkanaalbehandeling in twee of meer zittingen wordt uitgevoerd : de calciumhydroxide (CH)-pasta. In deze studie hebben we de desinfectie van dentinetubuli van vijf verschillende samenstellingen van calciumhydroxide-pasta's geëvalueerd met behulp van confocale laser scanning microscopie. De chemisch-fysische eigenschappen van de verschillende calciumhydroxide-pasta's, zoals pH, oplosbaarheid en calciumionafgifte werden geëvalueerd met behulp van een pH-meter, de Micro-CT en een atoomabsorptiespectrofotometer. De CH werd ofwel opgelost in gedestilleerd water of propyleenglycol. Bovendien werd de propyleenglycolgroep opgesplitst in drie groepen met ieder verschillende toevoegingen, ethanol extract van propolis, chloorhexidine of kamferparamonochloorfenol (CPMC). De CH met CPMC had een significant beter effect in vergelijking met CH met gedestilleerd water. In het algemeen waren de pasta's met propyleenglycol meer antimicrobieel, drongen beter door in de dentinetubuli en hadden betere chemisch-fysische eigenschappen.

In **Hoofdstuk 3** evalueren we het antimicrobiële effect van een innovatief-experimenteel wortelkanaalspoelmiddel met nano-zilverdeeltjes, in vergelijking met chloorhexidine (CHX) en de gouden standaard natriumhypochloriet (NaOCl). De spoelvloeistofen werden gedurende 5, 15 en 30 minuten getest op een *Enterococcus faecalis*-biofilm gekweekt op dentineschijfjes en in dentinetubuli van rundertanden. De data werden geanalyseerd met behulp van confocale laser scanning microscopie. NaOCl vertoonde het beste antibiofilm-effect, ook in de dentinetubuli, gevolgd door CHX en het spoelmiddel met nano-zilverdeeltjes. Het nieuw geteste spoelmiddel met nano-zilverdeeltjes bleek niet effectief te zijn in het verwijderen van *Enterococcus faecalis* biofilm in vergelijking met de andere geteste spoelmiddelen die tijdens de wortelkanaalbehandeling worden gebruikt.

In **Hoofdstuk 4** werd voor het eerst gebruik gemaakt van optische coherentie tomografie om het verwijderen van biofilm uit wortelkanaalmodellen met laterale morfologische structuren zoals een lateraal kanaal of isthmus te analyseren. NaOCl in verschillende concentraties werd met verschillende stroomsnelheden getest tijdens wortelkanaalirrigatie gevolgd door een laatste irrigatie met buffer met een hoge stroomsnelheid. Aanvullend werden van de gebruikte stroomsnelheden vloeistof-stroomprofielen in het wortelkanaalmodel gesimuleerd met behulp van een gevalideerde methode van Computational Fluid Dynamics. De stroomsnelheden werden gecorreleerd met het verwijderen van biofilm. De stroomsnelheid had meer invloed op de verwijdering van de biofilm dan de concentratie van het spoelmiddel, in gebieden met hogere stroomsnelheden werd meer biofilm verwijderd.

In **Hoofdstuk 5** hebben we de effectiviteit van vier verschillende irrigatieprotocollen getest in het verwijderen van biofilm uit een wortelkanaalmodel met laterale morfologische kenmerken zoals een lateraal kanaal of isthmus gelijkende structuren en uit humane dentinetubuli, met respectievelijk optische coherentie tomografie of confocale laser scanning microscopie als evaluatiemethode. De gebruikte irrigatieprotocollen waren handirrigatie met een bufferoplossing (controlegroep), een gemodificeerde zoutoplossing genaamd RISA, NaOCl en ultrasone activering van de bufferoplossing, allen gevolgd door een laatste irrigatie met een bufferoplossing met een hoge stroomsnelheid. Het mechanische effect van handirrigatie bleek een relevante factor te zijn voor het verwijderen van de biofilm. Ultrasone activering van de buffer bleek effectief te zijn wanneer het contactoppervlak biofilm-spoelmiddel klein was. In het dentine tubulesmodel werd naast het antimicrobiële effect, vijf dagen na de behandeling een rekolonisatie-analyse uitgevoerd. Deze analyse toonde aan dat de ‘post treatment remaining biofilm’ binnen een periode van vijf dagen zonder extra voeding in de dentinetubuli kon groeien. Het antibiofilm-effect van bufferoplossing, RISA en NaOCl werd ook getest op een biofilm gekweekt op schijfjes dentine. De dikte van de biofilm en de visco-elastische eigenschappen van de biofilm na de behandeling werden geëvalueerd, er was geen significant verschil aantoonbaar tussen de onderzochte spoelvloeistoffen.

In **Hoofdstuk 6** hebben we de invloed van de spoelvloeistof, de stroomsnelheid, de verversing van de spoelvloeistof en de applicatietijd op de verwijdering van biofilm uit de isthmus of lateraal kanaal gelijkende structuren in wortelkanaalmodellen geëvalueerd met behulp van optische coherentie tomografie. NaOCl en gedemineraliseerd water (controlegroep) werden gebruikt als spoelvloeistof met stroomsnelheden van 0,05 of 0,1 ml / sec. NaOCl in combinatie met een hoge stroomsnelheid resulteerde in meer biofilm verwijdering uit isthmusachtige structuren, terwijl voor het laterale kanaal de stroomsnelheid geen invloed had op de biofilmverwijdering. Verversing van de spoelvloeistof had geen significant effect op de verwijdering van biofilm.

Op basis van de resultaten verkregen uit het onderzoek besproken in de vorige hoofdstukken, behandelt **Hoofdstuk 7** de bevindingen en de verschillende gebruikte biofilm modellen en hun onderlinge samenhang. We geven ook een overzicht van de toekomstperspectieven op basis van de uitgevoerde onderzoeken om desinfectieprocedures tijdens de endodontische behandeling te optimaliseren. Dit zou mogelijk kunnen resulteren in een verbetering van de genezing van apicale parodontitis.

SUMÁRIO

O biofilme no sistema de canais radiculares representa um grande desafio durante a desinfecção na terapia endodôntica, principalmente aquele localizado em áreas de complexidade anatômica. O **Capítulo 1** fornece uma breve visão geral de como a infecção endodôntica começa e continua seu caminho no sistema de canais radiculares, onde as bactérias crescem e formam biofilmes. Além disso, mostra como as complexidades anatômicas do sistema de canais radiculares podem influenciar a desinfecção e os agentes antimicrobianos mais comuns utilizados na terapia endodôntica.

Assim, o **Capítulo 2** enfoca na medicação intracanal mais comumente utilizada entre sessões na terapia endodôntica: a pasta de hidróxido de cálcio. Assim, neste estudo, avaliamos a capacidade de descontaminação intratubular de cinco diferentes formulações de pastas de hidróxido de cálcio contra *Enterococcus faecalis* e a penetrabilidade destas pastas na dentina, por Microscopia Confocal de Varredura a Laser. As propriedades físico-químicas das diferentes pastas de hidróxido de cálcio, como pH, solubilidade e liberação de íons cálcio, foram avaliadas por um medidor de pH, o Micro CT e um espectrofotômetro de absorção atômica. Para a manipulação do hidróxido de cálcio água destilada e propilenoglicol foram utilizados como veículos. Além disso, o veículo propilenoglicol foi utilizado com três aditivos: extrato etanólico de própolis, clorexidina e paramonoclorofenol canforado (CPMC). A pasta com CPMC teve significativamente maior eficácia antimicrobiana quando comparada à pasta com água destilada. De maneira geral, as pastas que tinham propilenoglicol como veículo mostraram melhor eficácia antimicrobiana, penetrabilidade nos túbulos dentinários e apresentaram as melhores propriedades físico-químicas.

No **Capítulo 3**, avaliamos a eficácia antimicrobiana de um agente irrigante inovador com Nanopartículas de prata em comparação com a clorexidina e o irrigante padrão ouro hipoclorito de sódio. Os irrigantes foram testados por 5, 15 e 30 minutos em um biofilme de *Enterococcus faecalis* cultivado em blocos de dentina bovina e no interior de túbulos dentinários de dentes bovinos. As amostras foram analisadas por Microscopia Confocal de Varredura a Laser mostrando que o hipoclorito de sódio apresentou o melhor efeito antibiofilme tanto nos blocos de dente bovino quanto nos túbulos dentinários, seguido pela clorexidina e o irrigante com Nanopartículas de prata. Este último demonstrou ser ineficaz contra *Enterococcus faecalis* em comparação com os outros irrigantes testados.

No **Capítulo 4**, uma análise por Tomografia Óptica de Coerência da remoção de biofilme de modelos de canais radiculares de polidimetilsiloxano com complexidades anatômicas, com estruturas semelhantes a istmos e a canais laterais foi descrita pela primeira vez. A solução irrigadora hipoclorito de sódio foi utilizada em várias concentrações e em diferentes taxas de fluxo para irrigação do canal radicular, seguido de uma irrigação final com solução tampão em alta taxa de fluxo. Além disso, a velocidade do irrigante dentro do modelo de canal radicular foi medida em diferentes taxas de fluxo por meio de Fluidodinâmica Computacional e correlacionada com a remoção do biofilme. A taxa de fluxo de irrigante teve mais influência na remoção do biofilme do que sua concentração. A velocidade do irrigante influenciou a remoção do biofilme, pois em áreas com velocidades mais altas, mais biofilme foi removido. Uma alta taxa de fluxo também foi relacionada à maior velocidade de irrigação no interior do modelo.

No **Capítulo 5**, apresentamos uma comparação entre quatro diferentes protocolos de irrigação quanto à capacidade de remover biofilme do modelo de canal radicular com istmos e canais laterais e de túbulos dentinários, por meio de Tomografia Óptica de Coerência ou Microscopia Confocal de Varredura a Laser, respectivamente. Os protocolos de irrigação utilizados foram irrigação com seringa utilizando solução tampão (grupo controle), solução salina modificada chamada Risa, Hipoclorito de sódio e ativação ultrassônica da solução tampão, seguida de irrigação final com alta taxa de fluxo utilizando a solução tampão. O efeito mecânico da irrigação com seringa mostrou-se um fator relevante a ser observado no estudo da remoção de biofilme. A ativação ultrassônica do irrigante mostrou-se eficaz quando a superfície de contato do biofilme-irrigante era pequena. No modelo dos túbulos dentinários, além da eficácia antimicrobiana, uma análise de recolonização foi realizada cinco dias após o tratamento. Esta análise mostrou que o biofilme remanescente após o tratamento foi capaz de sobreviver e crescer no interior dos túbulos dentinários em um período de cinco dias sem qualquer nutrição extra. O efeito antibiofilme da solução tampão, Risa e Hipoclorito de sódio foi testado em um biofilme sobre discos de dentina. A redução da espessura do biofilme e as propriedades viscoelásticas do biofilme remanescente após o tratamento foram avaliadas não mostrando diferença entre as substâncias estudadas.

No **Capítulo 6**, investigamos ainda a influência do irrigante, taxa de fluxo, renovação do irrigante e tempo de exposição na remoção de biofilme dos modelos de canal radicular por meio de Tomografia Óptica de Coerência. Hipoclorito de sódio e água desmineralizada (grupo controle) foram utilizados como soluções irrigadoras levadas ao modelo de canal radicular com uma taxa de fluxo de 0,05 ou 0,1 mL/s. O hipoclorito de sódio e a maior taxa de fluxo apresentaram maior remoção de biofilme das estruturas semelhantes ao istmo, enquanto que, para o canal lateral a taxa de fluxo não teve influência. As amostras foram divididas em diferentes grupos de acordo com o número de renovações da solução irrigadora e essa variável não melhorou a remoção do biofilme. A análise de sequenciais renovações no mesmo biofilme mostrou um efeito cumulativo da irrigação no biofilme.

Com base nos resultados obtidos nos estudos descritos nos capítulos anteriores, o **Capítulo 7** discutiu os achados, diferentes modelos de biofilme utilizados e correlações entre eles. Além disso, fornece uma visão geral das perspectivas futuras com base nos estudos realizados, a fim de otimizar os procedimentos de desinfecção durante a terapia endodôntica, possivelmente levando a uma melhora na cicatrização da periodontite apical.

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